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SEPARATION SCIENCE AND TECHNOLOGY, 31(13), pp. 1843–1857, 1996

Supplied by U.S. Dept. of Agriculture
National Center for Agricultural
Utilization Research, Peoria, Illinois

Production of Tocopherol Concentrates by Supercritical Fluid Extraction and Chromatography*

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ABSTRACT

Supercritical fluid extraction (SFE) has been combined with supercritical fluid chromatography (SFC) in a preparative mode to develop a system for fractionating and enriching high value constituents contained in seed oil matrices. The system consists of an extraction step sequenced on-line with a sorbent filled column, which permits a SFE-enriched tocopherol fraction to be diverted onto the chromatographic column for further enrichment of the tocopherols. For the SFE stage, the tocopherol enrichment was optimized at 25 MPa and 80°C for soybean flakes and rice bran. However, total tocopherol recovery and enrichment was also found to be a critical function of the mass ratio of CO_2/seed charge. Approximately 60% of the available tocopherols in soyflakes can be recovered in the SFE step, yielding enrichment factors of 1.83–4.33 for the four tocopherol species found in soy-

* Names are necessary to report factually on available data; however, the USAD neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

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bean oil. Additional enrichment of tocopherol species can be realized in the SFC stage, ranging from 30.8 for delta-tocopherol to 2.41 for beta-tocopherol.

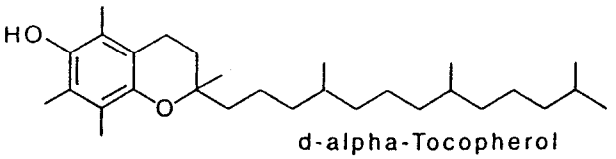
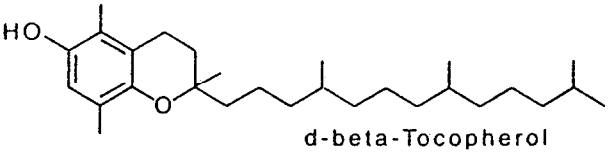
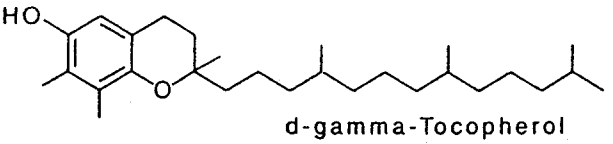
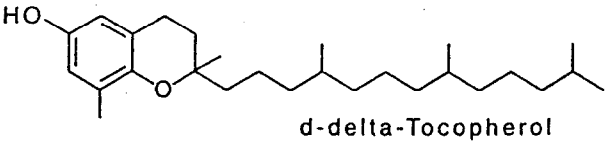
INTRODUCTION

It has been demonstrated that supercritical fluid extraction (SFE) is a facile and specific technique for the removal of many lipid species from a host of natural products (1, 2). This is due in part to the similarity of solubility parameters for many lipid species and the most commonly used supercritical fluid, CO_2, at specific pressures and temperatures utilized in the SFE (3, 4). Fractionation of a lipid extract by supercritical methods is much more difficult, due to the similarity in cohesive energy densities of individual lipid moieties (e.g., triglycerides, tocopherols, etc.), and SFE alone, cannot frequently resolve or significantly enrich a specific component from such complex mixtures.

Supercritical fluid chromatography (SFC) coupled with SFE provides a powerful adjunct technique to enrich various components from extracted mixtures. The efficacy of such a tandem methodology has been demonstrated frequently in analytical-scale separations (5, 6) and occasionally using "preparative" scale techniques (7, 8). These published methods, however, are quite diminutive in scale and utilize very expensive sorbent media that would be prohibitive for use in a large production scale SFE/SFC system. In this study we have designed and tested a supercritical fluid extraction and fractionation system that could be scaled into a production unit. The SFE stage was first optimized and then the SFC step. The chromatographic step makes use of a commodity sorbent, silica gel, to minimize production expenses, as well as to provide fractionation of tocopherols from coextracted seed oil components.

Tocopherol extraction and enrichment from natural products such as seed oils is practiced industrially. Molecular distillation is currently employed to obtain tocopherol extracts which find wide use as antioxidants in product formulations and as precursors in vitamin production (9, 10). The composition of such extracts will vary depending on their natural source (11), but frequently useful extracts are obtained from by-products, such as deodorizer distillate (12). Supercritical fluid technology has also been applied to extract tocopherols from such by-product mixtures (13–15) either by packed column techniques or by formation of more volatile derivatives to enhance their respective separation factors. Separation of the individual tocopherol species is at best difficult due to the similarities of their molecular weights and structures (Table 1). However, useful tocopherol mixtures can be enriched in vegetable oil bases which find application in industry (16).

TABLE 1
Tocopherols in Soybean Oil

Structural Formulas	Names	Empirical Formulas	Molecular Weights
	d-alpha-Tocopherol	$C_{29}H_{50}O_2$	430.69
	d-beta-Tocopherol	$C_{28}H_{48}O_2$	416.66
	d-gamma-Tocopherol	$C_{28}H_{48}O_2$	416.66
	d-delta-Tocopherol	$C_{27}H_{46}O_2$	402.64

Initial studies in our laboratory (17) have shown that a tocopherol-enriched fraction could be obtained from soyflakes by conducting the extraction at 24.1 MPa and 80°C. This finding is different from the Simplex predictions of Fisher (18), who showed that tocopherols could be preferentially extracted from rice bran at 27.6 MPa and 40°C. In this study we confirmed these previous results and extended our studies to additional substrates such as rice bran and wheat germ, coupling SFC with SFE for the enrichment of tocopherol mixtures from soybean oil.

EXPERIMENTAL

The experimental apparatus employed in these studies is shown in Fig. 1. Compressed carbon dioxide (Illinois Welding Supply, Bloomington, IL) was obtained from a cylinder initially at 6.2 MPa and further compressed to the desired extraction pressure via a booster compressor (Model AGT 52/152, Haskel, Inc., Burbank, CA). The extraction vessel and chromatographic column were contained in a modified gas chromatographic oven

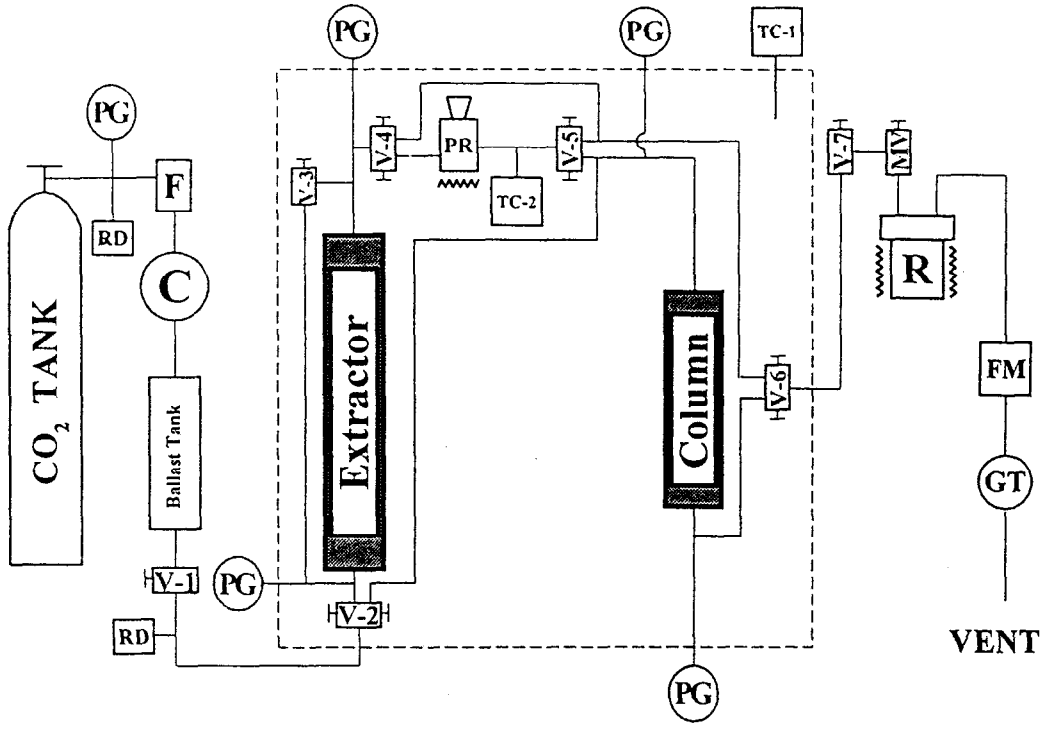


FIG. 1 Schematic of SFE/SFC processing system.

along with an equilibration coil to bring the compressed fluid to the extraction temperature. A pressure regulator (Model 26-1021-24-515, Tescom Corp., Elk River, MN) was also contained within the oven module and used in conjunction with the booster compressor to control the pressure on the chromatographic column. The flow rate from the extraction vessel and chromatographic column were regulated by a heated micrometering valve (No. 30VRMM48 12, Autoclave Engineers, Erie, PA), which also served to control the backpressure on the chromatographic column in conjunction with the pressure regulator. Collection of the extracts was made into a heated glass round-bottom flask (500 mL) outfitted with a special 24/40 adaptor to allow escape of the decompressed CO_2 stream. Gas volumes were measured with a dry test meter (Model DTM-115, American Meter Company, Philadelphia, PA) at ambient conditions. A similar system designed only for SFE has been described in the literature (19).

Extractions were performed by opening one side of the 2-way on/off valve (V-2) leading to the extraction vessel, along with the appropriate side of a 2-way valve (V-4) downstream from the extraction vessel. This routed the extraction fluid through the pressure regulator (PR) to the 2-

way valve (V-5) serving the column assembly, allowing the extract to be deposited at the top of the chromatographic column. Addition of the pressure regulator in the circuit allowed the pressure to be adjusted on the chromatographic column independent of the pressure on the extractor. The chromatographic stage of the operation was initiated by closing the previously opened side of the valve, V-2, serving the extractor, and opening the other side of this valve in conjunction with closing valve V-5. This diverts the supercritical fluid from the extraction vessel circuit through the column for eluting and further fractionating the lipid extract. Fractions can then be collected as a function of time (and mass of CO_2 utilized) by passing eluent through valves V-6 and V-7 to the micrometering valve into the receiver vessel.

Recovery of the remaining oil from the seed matrix in the extractor vessel can be effected by utilizing a different circuit in Fig. 1. In this sequence, the appropriate side of valve V-2 is opened and the extraction fluid is passed through the extraction vessel to valve V-4 which is opened so as to allow the fluid to bypass the pressure regulator to valve V-5, which is closed so that the fluid is diverted from passing onto the chromatographic column through the one open side of the 2-way valve V-6. This permits the recovery of the bulk of the remaining oil, containing enough tocopherol for protection from oxidation, after isolation of a fraction enriched in tocopherol having minimal oil content.

The extraction vessel consisted of a high pressure, 316 stainless steel tube, 61.0 cm in length having a 1.7-cm internal diameter, rated for use at 138 MPa (ambient conditions). This vessel could typically hold 70 g soybean flakes. The chromatographic column was made of identical material and was 20 cm in length $\times$ 1.7 cm internal diameter. Typically a charge of 16 g silica gel, 60–200 mesh (J.T. Baker Chemical Co., Phillipsburg, NJ) could be contained in the column using 10 μ porous end frits (Mott Metalurgic Co., Farmington, CT).

Although the optimization of the SFE and SFC stages will be discussed in greater detail later, a generic description of a typical experimental sequence is presented here. The SFE step was performed using a predetermined optimized quantity of CO_2, which for the SFE of approximately 70 g soyflakes was 1400 g (778 L CO_2 at ambient conditions). This quantity of CO_2 was used and the SFE terminated at this point to avoid further extraction of excess oil. The SFC stage was run by collecting fractions at equal volume intervals of CO_2 (500 L). This involved collecting approximately 6–8 individual fractions. The SFC column was maintained at approximately 5.5 MPa pressure (a low enough pressure to avoid eluting any of the extract from the column) during the SFE stage to concentrate the extract at the top of the silica gel column. In addition, this precaution

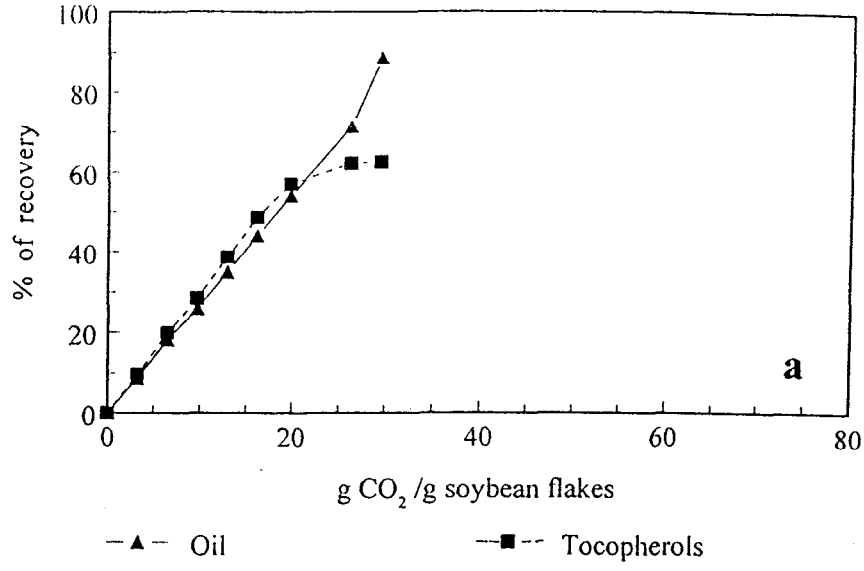


FIG. 2 Supercritical carbon dioxide extraction of three different seed matrices at 25 MPa and 40°C.

also avoided freezing up the valves from the Joule–Thompson expansion effect, as well as additional cost associated with recompression of the CO_2. The amount of remaining lipids on the SFC column, after treatment with SC-CO_2, was assessed gravimetrically by washing it with 300 mL ethyl acetate and evaporating the solvent with a stream of nitrogen.

Seed material for extraction was obtained from several sources and characterized for total oil and moisture content by standard methods (20). Soybean flakes were obtained internally within our laboratory, while rice bran samples were provided courtesy of Riceland Food, Inc. (Stuttgart, AR). Flaked wheat germ was provided by Dr. Dale Eustace (Department of Science & Industry, Kansas State University, Manhattan, KS). All substrates were stored at −20°C.

Analysis of tocopherol content and individual tocopherol species in the extract and oil samples was accomplished by HPLC (21). A Spectra Physics pump (Model SP-8800, San Jose, CA) was used in conjunction with a 5-μ silica, 250 × 4.6 mm column (Alltech Associates, Inc., Deerfield, IL). Chromatography was conducted using a *n*-hexane/2-propanol (99.5/0.5 vol%) mobile phase in an isocratic mode. Solute detection was accomplished by using a fluorescent detector (Model LC 304, Linear Instruments, Reno, NV); excitation wavelength, 290 nm; emission wavelength, 330 nm. The HPLC column was kept at 35°C with the aid of a column heater (Model 1250425, Bio-Rad, Inc., Richmond, CA). All HPLC analy-

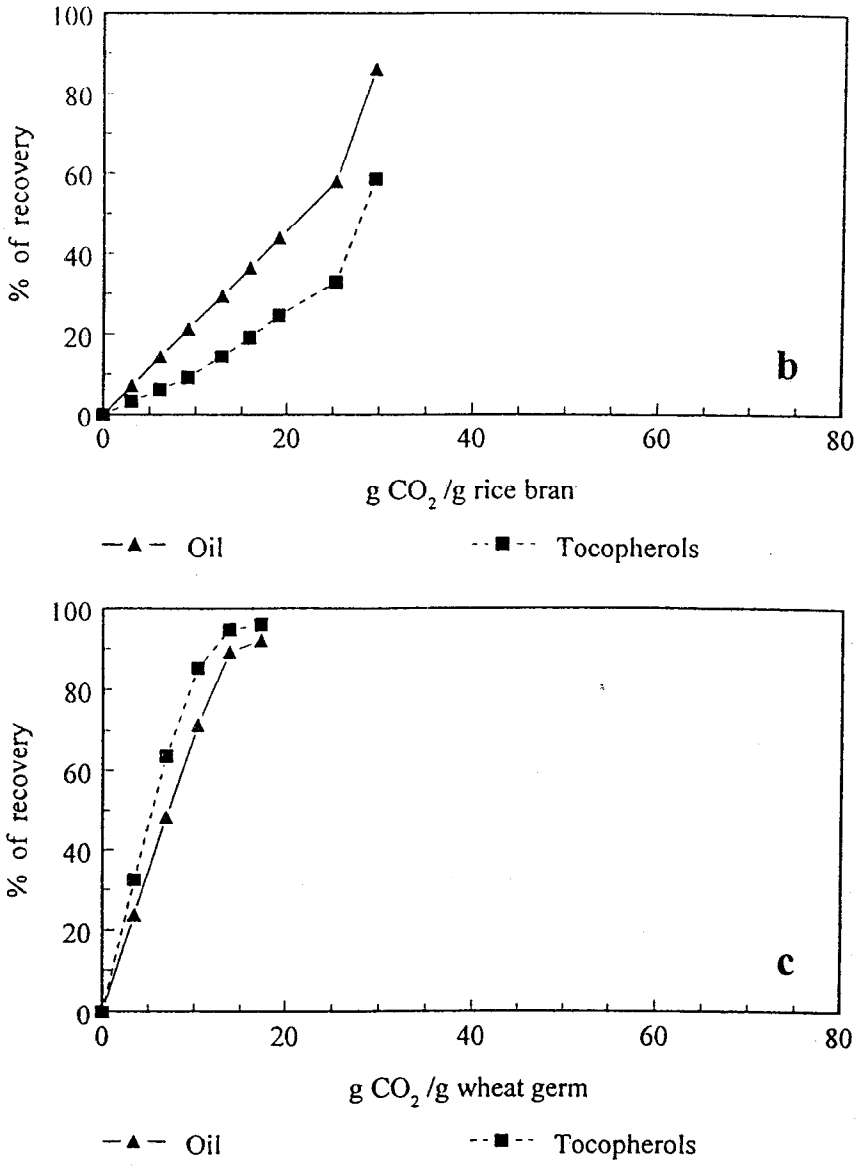


FIG. 2 Continued

ses were performed at a flow rate of 1.0 mL/min. Tocopherol standards were obtained from Matreya, Inc., Pleasant Gap, PA.

RESULTS AND DISCUSSION

To assure proper operation of the constructed SFE/SFC unit, it was necessary to conduct experiments for both the SFE and SFC stages in order to optimize the process. Experiments for the extraction step were

run at 25 MPa and 40°C and 80°C based on earlier extraction results (17), and 70 MPa at 80°C for all three seed matrices. SFC sequences were run at 25MPa and 40°C, 60°C and 80°C; and at 70 MPa and 80°C. In all experiments the CO_2 flow rate for both the extraction and SFC steps was kept at approximately 5 L/min as measured at ambient conditions.

Extraction curves for the percent recovery of both tocopherols and total oil from soyflakes, plotted as a function of g-CO_2/g-seed utilized, are shown in Figs. 2(a–c) for extractions conducted at 25 MPa and 40°C. The recoveries are based on total available tocopherol content in a specific seed oil and the oil content of the seed charge. Figure 2(a) shows that as SC-CO_2 is passed through the seed bed in the extractor, there is hardly any fractionation of tocopherols from the bulk oil under these extraction conditions. At g-CO_2/g-seed charges in excess of 20, the extraction pressure was raised to 70 MPa to facilitate removal of all of the oil from the seed bed. This change clearly produces a more dilute tocopherol extract, the opposite of what is desired.

A similar result to this was recorded for the SFE of rice bran under these same experimental conditions (Fig. 2b), except that more oil is preferentially extracted relative to tocopherols/tocotrienols right from the start of the extraction. For the SFE of wheat germ, it appears that both tocopherols and wheat germ oil are extracted at similar rates, resulting in no improvement in tocopherol/oil separation (Fig. 2c). At 25 MPa and 80°C

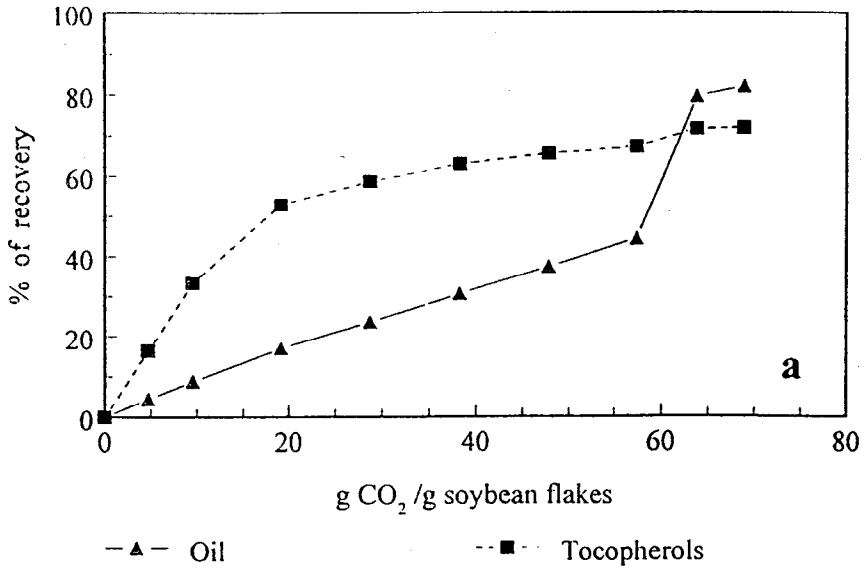


FIG. 3 Supercritical carbon dioxide extraction of three different seed matrices at 25 MPa and 80°C.

there is a recorded enhancement in the recovery of tocopherols relative to extracted oil over an extended g-CO_2/g-seed range (Figs. 3a–c). The degree of selective partitioning of the tocopherols under these extraction conditions varies with the type of seed matrix being extracted, the most significant enhancement of tocopherols being recorded for soyflakes (Fig. 3a). The recovery differences in this case are the largest between the tocopherols and oil of the three seed matrices examined. At a g-CO_2/g-seed ratio of about 60 (50 in the case of wheat germ), the extraction pres-

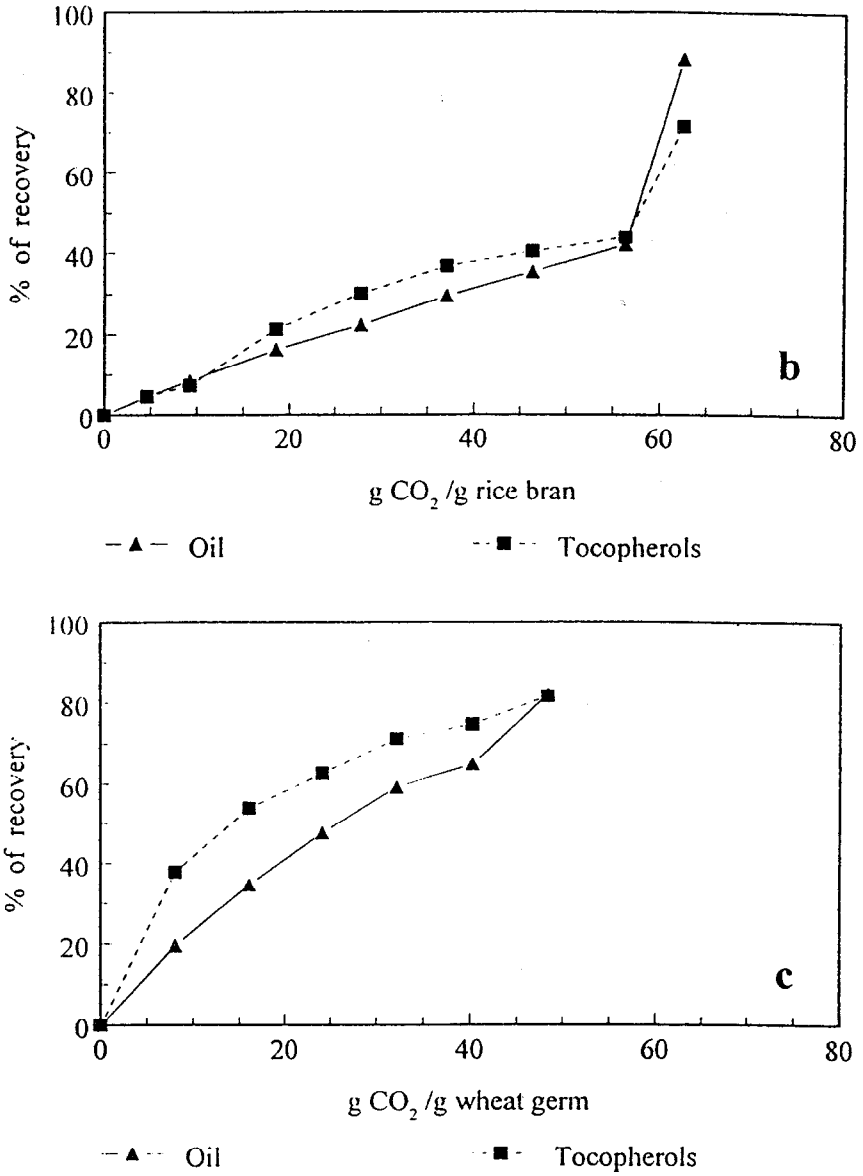


FIG. 3 Continued

sure was raised to 70 MPa to remove the oil from the seed bed, since recovery of tocopherols relative to extracted oil had begun to diminish at this point. Therefore it would appear that concentrated tocopherol extracts can best be obtained in the cited g-CO_2/g-seed range without the expense and labor associated with further processing time.

Higher pressure (70 MPa) and temperature (80°C) extraction conditions which are optimal for total oil removal from the seed matrix (22, 23) typically yield a result as shown in Fig. 4 for soybean oil extraction. Whereas these extraction conditions are clearly unfavorable for producing the desired separation, they can be used to advantage as noted previously for the total removal of oil from the seed matrix.

Figure 5 illustrates better the fractionation and enrichment of tocopherols from a seed oil matrix as well as the role of coextracted oil on the concentration of the tocopherols in the extracted fractions. The histograms presented in Fig. 5 (top) show the concentration of tocopherols relative to the oil in the collected fractions in comparison with the individual tocopherol concentrations in the starting oil at 25 MPa and 80°C. Clearly, considerable concentration of the tocopherols has been achieved in the early fractions, and they vary somewhat with the identity of the specific tocopherol specie. This concentration effect is partly due to the selective solubility and rates of removal of the tocopherols from the various seed matrices, particularly relative to the seed oil. Similar results were obtained by Zhao et al. (24) for fractions obtained during the SFE of rice bran oil. Figure 5 (bottom) shows the recovery of total oil from

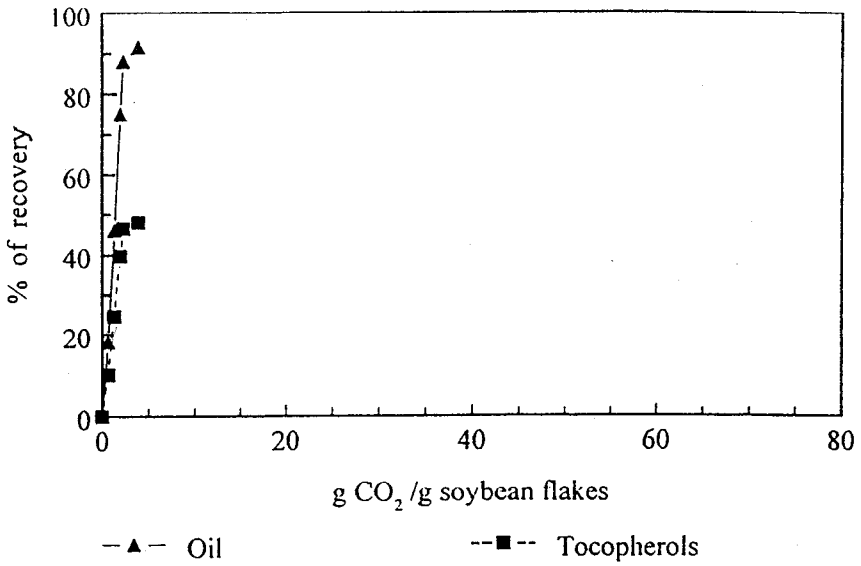


FIG. 4 Supercritical carbon dioxide extraction of soyflakes at 70 MPa and 80°C.

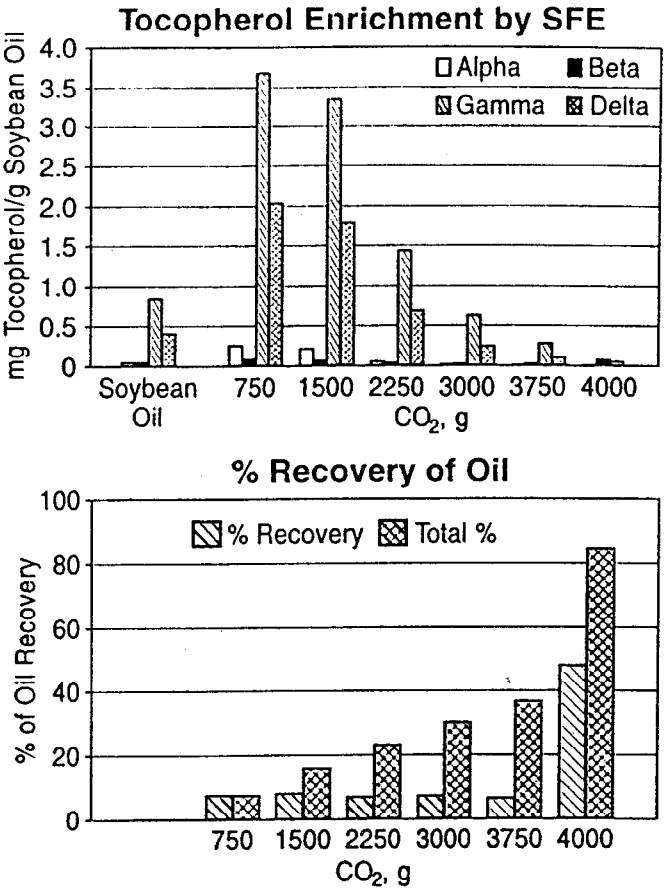


FIG. 5 Tocopherol enrichment and oil recovery during the SFE of soybean flakes.

the seed charge on a cumulative basis as the extraction proceeds. A comparison of the results in Fig. 5 (top) with those in Fig. 5 (bottom) clearly indicates that carrying the preliminary extraction stage beyond a certain point is counterproductive.

Tocopherol composition of the collected fractions (every 500 L CO_2 at ambient conditions) during SFC fractionation was measured on soyflake extracts by determining the individual tocopherol content by HPLC. From these experiments it was concluded that the conditions of 25 MPa and 40°C were sufficient to optimize the fractionation and enrichment of tocopherols during the SFC step. Analysis of the fractions from SFC step for individual tocopherol content indicated the preferential enrichment of some tocopherols during the course of their elution from the column. As shown in Fig. 6, alpha- and beta-tocopherol were isolated in the first fraction collected, while fractions 2 and 3 were found to contain varying amounts of gamma- and delta-tocopherols. Fractions 4–6 consisted mostly

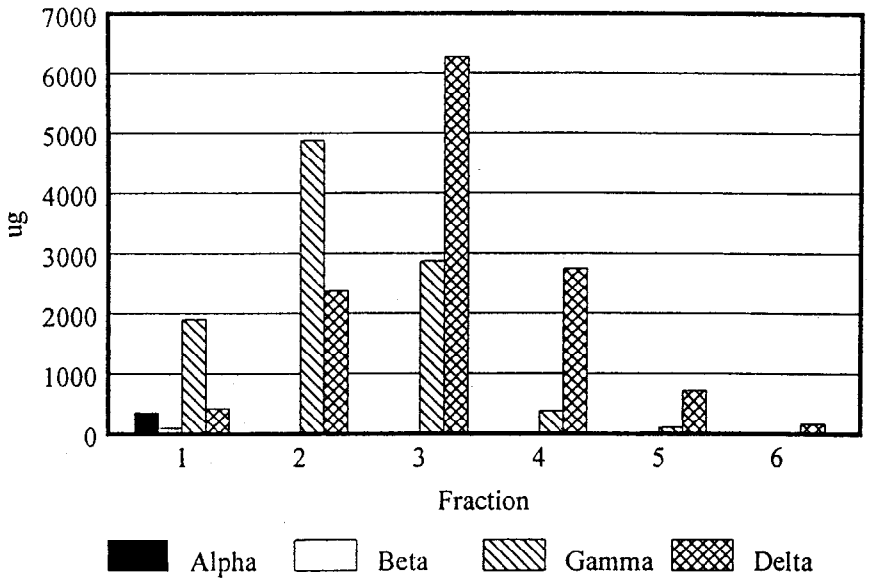


FIG. 6 Tocopherol content of collected SFE/SFC fractions.

of delta-tocopherol. Obviously this selective chromatographic fractionation of the tocopherol isomers can be used as an additional way of isolating specific tocopherols.

Table 2 summarizes the enrichment of the individual tocopherols initially during the SFE stage (25 MPa, 80°C) and by also using a combination of SFE/SFC, with the SFC step conducted at 25 MPa and 40°C. The reported enrichments are for tocopherols extracted and chromatographed from soyflakes, and the reported values are the mean values from triplicate runs relative to the individual tocopherol content initially found in the soybean oil. An enrichment factor of approximately 4 is obtained for three of the tocopherols during the initial SFE step. Even larger enrichments

TABLE 2
Enrichment Factors for Individual Tocopherols after SFE/SFC Relative to Starting Concentration in Soyflakes

Tocopherol	SFE stage	SFC stage
Alpha	4.33	12.1
Beta	1.83	2.4
Gamma	3.94	15.0
Delta	3.75	30.8

TABLE 3
Percent Recovery of Available Tocopherol from the SFE and SFC Stages

Tocopherol	SFE stage	SFC stage
Gamma	62.7	75.7
Delta	60.2	87.4

of the tocopherols can be achieved by combining SFC with the SFE stage. As indicated in Table 2, this can range from a 30-fold enrichment for the delta-tocopherol to values of 15 and 12 for the gamma- and alpha-tocopherol, respectively, relative to their concentrations in the beginning soyflakes. This represents an 8.2, 3.8, and 2.8 enrichment of the delta-, gamma-, and alpha-tocopherols, respectively, during the SFC stage. Beta-tocopherol, on the other hand, experiences hardly any enrichment from the chromatographic stage. The SFE/SFC-derived enrichments reported here are significantly higher than the enrichments reported in the literature using only SFE (13).

The percent mass recoveries of the two major tocopherols in soybean oil relative to the total available tocopherol in the soyflakes from the SFE stage are reported in Table 3. Both gamma- and delta-tocopherol were recovered to an approximately 60% level from the soyflakes under the processing conditions reported above. Complete recovery of all of the tocopherols from the soybean flakes is actually undesirable, since their presence retards the oxidation of the oil (9). Tocopherol recoveries from the silica gel were 76 and 87%, respectively, for the gamma and delta isomers, based on the mass of extract from the SFE stage that was transferred onto the chromatographic column. These recoveries are very similar to those obtained by Shishikura et al. (25) who used silicic acid as an adsorbent to improve the purity of their supercritical fluid extract derived from esterified deodorizer distillate sludge. Recoveries for alpha- and beta-tocopherols were not computed since they were eluted in the initial SFC fraction and they occur at extremely low levels naturally in soybean oil.

CONCLUSIONS

This study has demonstrated that enrichment of tocopherols from seed oil matrices can be achieved by utilizing SFE in tandem with a SFC fractionation step. The experimental system has been designed to allow for relatively easy scale-up to plant-size operation, using an inexpensive,

commodity sorbent for fractionating the SFE product. SFE has been optimized for several seed types and has been shown to be substrate-dependent.

One facet of this study deserves further comment. As was noted in the Introduction, SFE from a natural product matrix may yield an "effective" solubility level that is quite different from that obtained for the neat compound. Therefore optimal extraction conditions may be different from those predicted by solubility studies on the pure compound (26) and show a matrix dependence. This is confirmed in Figs. 2(a–c) and 3(a–c) where the tocopherol recoveries differ from one seed type to another.

The generic construction and operation of the described system should be applicable for the recovery of other natural products from a variety of matrices. Viable processing options that can be integrated into the system include other sorbents and perhaps cosolvent addition into the SC-CO_2 during the SFC stage to further enhance recoveries. These options are currently being explored in our laboratory.

ACKNOWLEDGMENTS

The assistance of the Analytical Chemistry Support Group in measuring proximate values of the seed oil samples is gratefully acknowledged. We also thank G. List (NCAUR) for procuring some of the oilseed stocks used in this study.

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Received by editor October 16, 1995